Toxicity of Thiophenes from *Echinops transiliensis* (Asteraceae) against *Aedes aegypti* (Diptera: Culicidae) Larvae

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Structure–activity relationships of nine thiophenes, 2,2′:5′,2″-terthiophene (**1**), 2-chloro-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-1-yl acetate (**2**), 4-(2,2′-bithiophen-5-yl)but-3-yne-1,2-diyl diacetate (**3**), 4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yne-1,2-diyl diacetate (**4**), 4-(2,2′-bithiophen-5-yl)-2-hydroxybut-3-yn-1-yl acetate (**5**), 2-hydroxy-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-1-yl acetate (**6**), 1-hydroxy-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-2-yl acetate (**7**), 4-(2,2′-bithiophen-5-yl)but-3-yne-1,2-diol (**8**), and 4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yne-1,2-diol (**9**), isolated from the roots of *Echinops transiliensis*, were studied as larvicides against *Aedes aegypti*. Structural differences among compounds **3**, **5**, and **8** consisted in differing AcO and OH groups attached to C(3″) and C(4″), and resulted in variations in efficacy. Terthiophene **1** showed the highest activity (LC_{50}, 0.16 μg/ml) among compounds **1**–**9**, followed by bithiophene compounds **3** (LC_{50}, 4.22 μg/ml), **5** (LC_{50}, 7.45 μg/ml), and **8** (LC_{50}, 9.89 μg/ml), and monothiophene compounds **9** (LC_{50}, 12.45 μg/ml), **2** (LC_{50}, 14.71 μg/ml), **4** (LC_{50}, 17.95 μg/ml), **6** (LC_{50}, 18.55 μg/ml), and **7** (LC_{50}, 19.97 μg/ml). These data indicated that *A. aegypti* larvicidal activities of thiophenes increase with increasing number of thiophene rings, and the most important active site in the structure of thiophenes could be the tetrahydro-thiophene moiety. In bithiophenes, **3**, **5**, and **8**, *A. aegypti* larvicidal activity increased with increasing number of AcO groups attached to C(3″) or C(4″), indicating that AcO groups may play an important role in the larvicidal activity.

Introduction. – Mosquitoes transmit pathogens that cause serious human diseases including malaria, Japanese encephalitis, yellow fever, dengue, and filariasis. The urban-adapted *Aedes aegypti* mosquito has become widely distributed across tropical and subtropical latitudes. It emerged from Africa during the slave trade in the 15th through 19th centuries, spread to Asia through commercial exchanges in the 18th and 19th centuries, and has spread globally with the advent of increased travel and trade in the past 50 years [1]. Dengue fever is by far the most rapidly expanding vector borne disease with an estimation of 50–100 million infections occurring annually [2]. Insecticides from various chemical groups are the basic tools used for management of mosquito populations. Due to continuous use of insecticides, mosquitoes have

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developed resistance against these chemicals, and vector population management has become difficult [3]. The use of synthetic pesticides also poses serious human health and environmental concerns [4]. Hence, natural product-based products have gained special importance as potential new pesticides.

Plants are a rich source of bioactive compounds [5]. Thiophenes, derived from *Echinops* and *Tagetes* species [6][7], are reported to have strong toxicities against insects [8], plants [9], and plant pathogenic fungi [10]. Recently, thiophene compounds **1**, **2**, and **9** (*cf. Fig. 1*), which exhibit toxicity against the Formosan subterranean termite (*Coptotermes formosanus*), have been isolated from *E. transiliensis* collected from the Republic of Kazakhstan [11][12]. However, phytochemical studies of *E. transiliensis* remained limited. Compound **1** and related thiophenes have been reported to be toxic against mosquito (*A. aegypti* and *A. atropalpus*) larvae [13–15], whereas structure–activity relationships on thiophenes as larvicides have not been systematically studied. In our continuing study of the thiophenes from *Echinops* species [12][16], we isolated

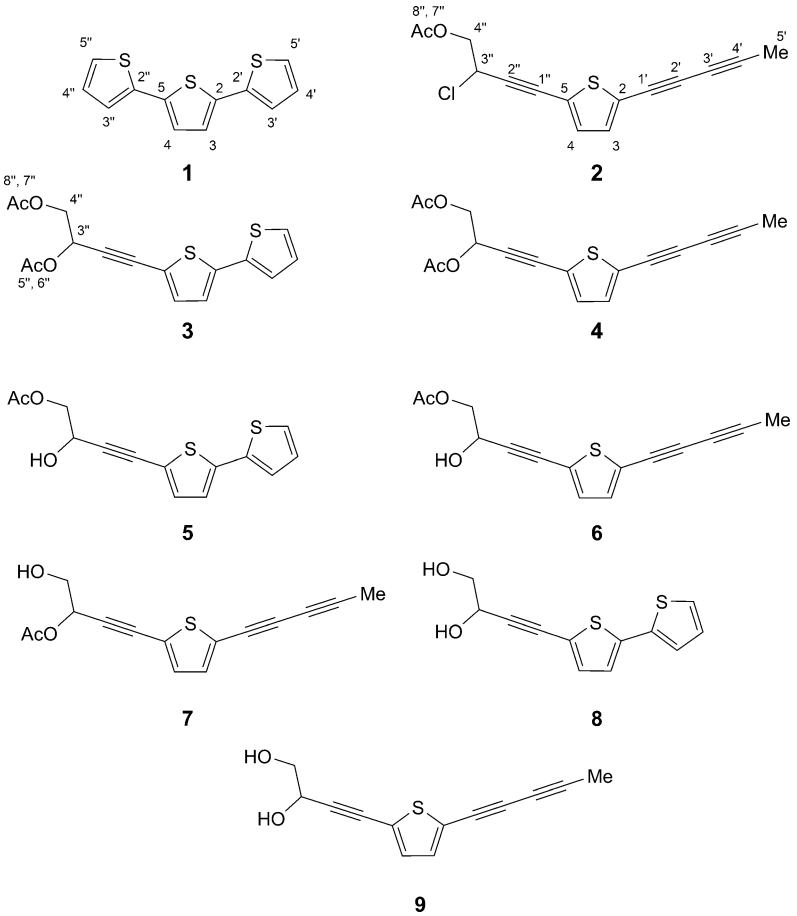

Fig. 1. *Structures of compounds* **1**–**9**

six known thiophene compounds **3**–**8** (*Fig. 1*). Herein, we describe the isolation and structure elucidation of thiophene compounds **3**–**8**, and the structure–activity relationships of compounds **1**–**9** as larvicides against *A. aegypti*.

Results and Discussion. – *Isolation and Identification of Compounds* **3**–**8**. To isolate thiophenes from the roots of *E. transiliensis*, the EtOH extract was subjected to normal-phase column chromatography followed by normal-phase HPLC to yield compounds **2**–**8** (*Fig. 2*). 2,2′:5′,2″-terthiophene (**1**) and 4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yne-1,2-diol (**9**) have been previously isolated from the EtOH extracts [12]. To further isolate the thiophenes from the roots of *E. transiliensis*, the CH_2Cl_2 extract was subjected to normal-phase column chromatography, followed by normal-phase HPLC to yield compounds **3**–**9** (*Fig. 2*). Compounds **1** and **2** have been previously isolated also from the CH_2Cl_2 extracts [11]. The gross structures of compounds **3**, **4**, and **6**–**8** (*Fig. 1*) were elucidated by analyses of EI-MS, and ^{1}H- and ^{13}C-NMR data, which were in complete agreement with literature data of 4-(2,2′-bithiophen-5-yl)but-3-yne-1,2-diyl diacetate (**3**) [17], 4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yne-1,2-diyl diacetate (**4**) [6], 2-hydroxy-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-1-yl acetate (**6**) [6], 1-hydroxy-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-2-yl acetate (**7**) [6], and 4-(2,2′-bithiophen-5-yl)but-3-yne-1,2-diol (**8**) [18], respectively. Compound **5** was analyzed by EI-MS, and ^{1}H- and ^{13}C-NMR, and identified as 4-(2,2′-bithiophen-5-yl)-2-hydroxybut-3-yn-1-yl acetate. This is the first report on the presence of compounds **3**–**8** in *E. transiliensis*.

Structure–Activity Relationships of Compounds **1**–**9** *as Toxicants against* A. aegypti *Larvae.* It has been reported that compound **1** showed toxicity against *A. aegypti* and blackfly (*Simulium vittatum*) larvae, adult nematodes (*Caenorhabditis elegans*) [8], and adult termites (*C. formosanus*) [11], while bithiophenes or monothiophenes have not been systematically studied as toxicants against *A. aegypti* larvae. Furthermore, there are no previous reports on the precise relationship between the number of thiophene rings present and the toxic activity against *A. aegypti* larvae. Compound **1** belongs to the class of compounds designated as terthiophene, compounds **3**, **5**, and **8** are designated as bithiophenes, and compounds **2**, **4**, **6**, **7**, and **9** are monothiophenes (*Fig. 1*). The structural difference between compounds **3** and **4** consists in differing structural motifs at C(2). At C(2) of **3**, there is a thiophen-2-yl moiety, while C(2) of **4** carries a penta-1,3-diyn-1-yl group. The structural difference between compounds **5** and **6** consists in differing structural motifs at C(2). At C(2) of compound **5**, a thiophen-2-yl is attached, while C(2) of **6** bears a penta-1,3-diyn-1-yl moiety. Similar differences were observed between compounds **8** and **9**: C(2) of compound **8** is attached to a thiophen-2-yl moiety, while that of **9** is attached to a penta-1,3-diyn-1-yl group. Compound **1** showed the highest activity among the compounds tested in this study (*Table 1*). Based on non-overlapping 95% confidence intervals (*CI*s), **3**, **5**, and **8** with a thiophen-2-yl substituent at C(2) showed significantly higher activities than compounds **4**, **6**, and **9** with penta-1,3-diyn-1-yl groups at C(2), respectively. This was a clear indicator that larvicidal activities of thiophenes against *A. aegypti* increased with increasing number of thiophene rings, and the most important active site in the structure of thiophenes could be the attached thiophene skeleton. Structural differences between **3**, **5**, and **8** consist in functional groups attached to C(3″) and C(4″) (*Fig. 1*). AcO groups are

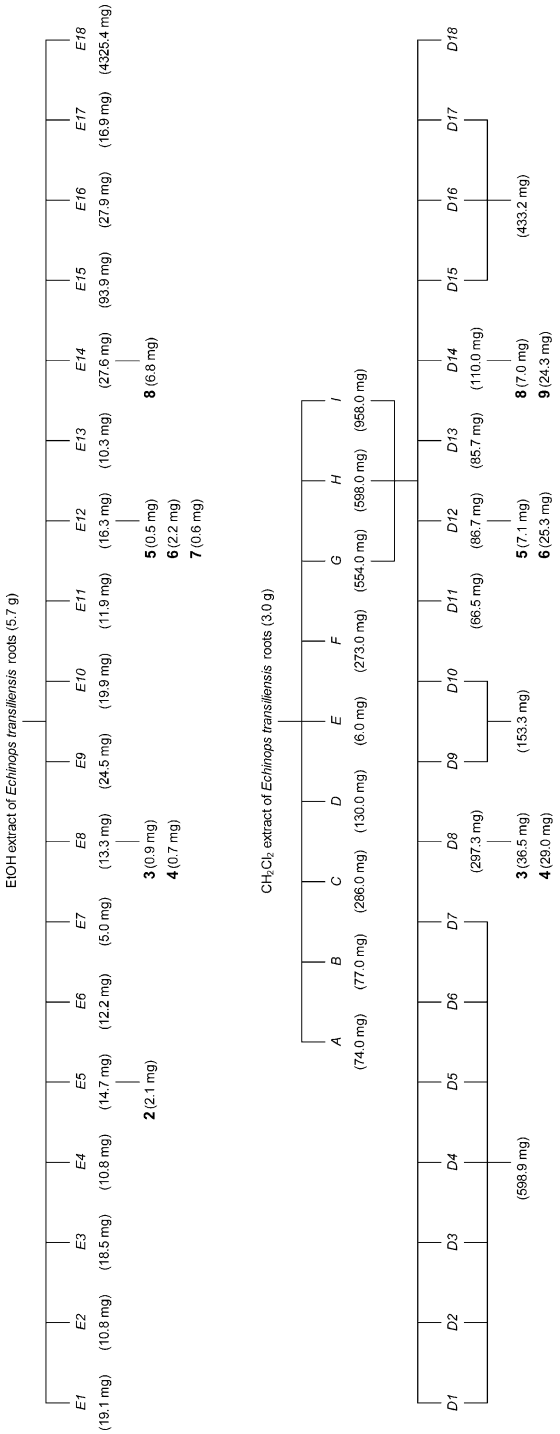

Fig. 2. *Schematic representation of the separation of the EtOH and CH_2Cl_2 extracts from the roots from* Echinops transiliensis

attached to C(3″) and C(4″) of compound **3**. OH and AcO groups are attached to C(3″) and C(4″), respectively, of compound **5**. OH Groups are attached to C(3″) and C(4″) of compound **8**. Compound **3** exhibited the highest activity, followed by **5** and **8** (*Table 1*). Hence, in bithiophenes, larvicidal activity appears to increase with an increasing number of AcO groups attached to C(3″) or C(4″), indicating that the AcO groups may play important roles in improving the larvicidal activity. In contrast, such a structure–activity relationship was not found among monothiophenes.

Contribution of Compounds **1**–**9** to *Larvicidal Activity of CH_2Cl_2 Extract* of A. aegypti. To evaluate the contribution of individual compounds to the larvicidal activity of the CH_2Cl_2 extracts, concentrations of compounds **1**–**9** in CH_2Cl_2 extracts were studied. Compound **1** had a lower concentration in the extract (1.00 g/kg) than **2** (7.12 g/kg) and higher concentration than other compounds (*Table 2*). Compound **1** which was *ca.* 92 times more active than compound **2** had *ca.* 1/7 of the concentration of **2**. As a result, contribution indices (concentration of compound in extract/LC_{50} and concentration of compound in extract/LC_{90}) of compound **1** were the highest among **1**–**9** (*Table 3*). Thus, compound **1** might be the most important constituent of the CH_2Cl_2

Table 1. *Toxicity of Compounds* **1**–**9** *Isolated from* Echinops transiliensis *Roots against* Aedes aegypti *Larvae*

Compound	LC_{50}	95% *CI*[a])	LC_{90}	95% *CI*[a])	χ^2	*DF*
1	0.16	0.15–0.18	0.25	0.22–0.30	51.69	73
2	14.71	12.74–17.06	25.85	21.55–34.40	50.28	38
3	4.22	3.61–4.89	11.19	9.15–14.73	99.87	73
4	17.95	14.66–22.46	76.58	53.47–131.86	72.70	48
5	7.45	6.53–8.54	16.10	13.42–20.68	98.91	73
6	18.55	16.23–21.25	29.76	25.32–38.67	42.37	73
7	19.97	16.44–24.38	32.01	25.88–49.06	21.88	73
8	9.89	7.82–13.01	29.88	20.77–54.97	46.23	73
9	12.45	11.09–14.02	21.86	18.74–27.32	69.56	48
Permethrin[c])	0.0034	0.0030–0.0038				

[a]) LC_{50} and LC_{90} values are given in μg/ml 95% confidence interval (*CI*). [b]) *DF*, Degree of freedom. [c]) Positive standard; purity, 46.1 and 53.2% for *cis* and *trans*, respectively.

Table 2. *Concentration of Compounds* **1**–**9** *in Extract and Root*

Compound	Weight/extract weight [g/kg]	Weight/root weight [mg/kg]
1	1.00 ±0.0619[a])	25.1 ±1.15
2	7.12 ±0.373	179 ±6.52
3	0.501 ±0.0626	12.6 ±1.41
4	0.645 ±0.0490	16.2 ±0.987
5	0.0337±0.00872	0.843±0.21
6	0.0859±0.00367	2.16 ±0.0670
7	0.0327±0.00462	0.822±0.115
8	0.293 ±0.00652	7.37 ±0.0781
9	0.549 ±0.00315	1.38 ±0.0730

[a]) Means ± SE of results from three extractions.

Table 3. *Contribution Index of Compounds* **1**–**9** *in* Aedes aegypti *Larvicidal Activity of CH_2Cl_2 Extracts*

Compound	Concentration/LC_{50} [mg/kg]/[mg/ml]	Concentration/LC_{90} [mg/kg]/[mg/ml]
1	6256	4004
2	484	275
3	119	45
4	36	8
5	5	2
6	5	3
7	2	1
8	30	10
9	4	3

extracts in *A. aegypti* larvicidal activity. The CH_2Cl_2 extracts also exhibited high toxicity against *A. aegypti* larvae (LC_{50}, 3.21 mg/l; LC_{90}, 6.81 mg/l). The LC_{50} value of permethrin was 0.0034 mg/l. In the screening bioassay, permethrin was used at a dose of 0.025 ppm which led to 100% larval mortality.

Results from this study suggest that CH_2Cl_2 extracts or crude compound **1** fraction from CH_2Cl_2 extracts of the roots of *E. transiliensis* could be important tools for *A. aegypti* larval management. Further research is needed to conduct more comprehensive bioassays to explore the ways to effectively use these compounds in mosquito population management programs.

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Experimental Part

General. Colum chromatography (CC): *Biotage Isolera One* flash purification system (*Biotage*, SE-Uppsala). High-performance liquid chromatography (HPLC): *Agilent 1100* HPLC system (*Agilent*, Palo Alto, CA). ^{1}H- and ^{13}C-NMR spectra: *Varian Unity Inova AS600* spectrometer (*Varian*, Palo Alto, CA). EI-MS: *Varian CP-3800* gas chromatograph coupled to a *Varian Saturn 2000* mass spectrometer (*Varian*, Palo Alto, CA).

Plant Material. The roots of *Echinops transiliensis* Golosk. were collected at the flowering stage on March 12, 2004, on the slopes of mountains near Djandosov village in Zailiysky Alatau, Kazakhstan. A voucher specimen No. 9442/25-1972 was deposited with the Institute of Botany and Phytointroduction herbarium, Almaty, Republic of Kazakhstan.

Isolation of Compounds **2**–**8** *from the EtOH Extracts.* Powdered roots (0.5 kg) of *E. transiliensis* were extracted with 3.5 l of EtOH for 24 h at r.t. providing 10.7 g of extracts. A portion of the EtOH extract (5.7 g) was subjected to a normal-phase CC (*Flash 25+M*, *KP-Sil*, 25 × 150 mm, 40 g; *Biotage*; step gradient (step *1*, hexane/acetone from 100 : 0 to 80 : 20, 1200 ml; step *2*, hexane/acetone from 80 : 20 to 0 : 100; 600 ml)) to afford *Fr. E1* (19.1 mg), *Fr. E2* (10.8 mg), *Fr. E3* (18.5 mg), *Fr. E4* (10.8 mg), *Fr. E5* (14.7 mg), *Fr. E6* (12.2 mg), *Fr. E7* (5.0 mg), *Fr. E8* (13.3 mg), *Fr. E9* (24.5 mg), *Fr. E10* (19.9 mg), *Fr. E11* (11.9 mg), *Fr. E12* (16.3 mg), *Fr. E13* (10.3 mg), *Fr. E14* (27.6 mg), *Fr. E15* (93.9 mg), *Fr. E16* (27.9 mg), *Fr. E17* (16.9 mg), and *Fr. E18* (4325.4 mg; MeOH-wash fraction). Previously, *2,2′:5′,2″-terthiophene* (**1**) and compound **9** were isolated from *Fr. E3* and *Fr. E14*, resp. [12]. *Fr. E5* was further

separated by a normal-phase CC (*Flash 12 + M*, *KP-Sil* (12 × 150 mm, 9 g, *Biotage*); hexane/acetone from 100 : 0 to 90 : 10, 360 ml) to yield *2-chloro-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-1-yl acetate* (**2**; 2.1 mg, 0.00079%). *Fr. E8* was further separated by semi-prep. normal-phase HPLC (*Luna 5 µm Silica (2) 100 Å*, *Phenomenex*, 10 × 250 mm; hexane/^{i}PrOH, 99.2 : 0.8; flow rate, 4.7 ml/min; UV detection at 320 nm) to yield **3** (t_R 14.0 min; 0.9 mg, 0.00034%) and compound **4** (t_R 14.9 min; 0.7 mg, 0.00026%). *Fr. E12* was further separated by semi-prep. normal-phase HPLC (*Luna 5 µm Silica (2) 100 Å*, *Phenomenex*, 10 × 250 mm; hexane/^{i}PrOH, 97.0 : 3.0; flow rate, 4.7 ml/min; UV detection at 320 nm) to yield **5** (t_R 17.0 min; 0.5 mg, 0.00019%), **6** (t_R 18.9 min; 2.2 mg, 0.00083%), and **7** (t_R 23.8 min; 0.6 mg, 0.00023%). *Fr. E14* was further separated by semi-prep. normal-phase HPLC (*Luna 5 µm Silica (2) 100 Å*, *Phenomenex*, 10 × 250 mm; hexane/^{i}PrOH, 95.0 : 5.0; flow rate, 4.7 ml/min; UV detection at 320 nm) to afford compound **8** (t_R 22.1 min; 6.8 mg, 0.00255%).

Isolation of Compounds **3**–**9** *from the CH_2Cl_2 Extracts.* Powdered roots (0.5 kg) of *E. transiliensis* were extracted with 3.4 l of CH_2Cl_2 for 24 h at r.t. providing 8.2 g of CH_2Cl_2 extracts. A portion of the CH_2Cl_2 extract (3.0 g) was subjected to a normal-phase CC (*Flash 40 + M*, *KP-Sil*, 40 × 150 mm, 100 g, *Biotage*) using a step gradient (step *1*, hexane/AcOEt from 100.0 : 0.0 to 90.0 : 10.0, 1101 ml; step *2*, hexane/acetone from 90.0 : 10.0 to 70.0 : 30.0, 600 ml; step *3*, 70.0 : 30.0 to 0.0 : 100.0, 600 ml) to furnish *Fr. A* (74.0 mg), *Fr. B* (77.0 mg), *Fr. C* (286.0 mg), *Fr. D* (130.0 mg), *Fr. E* (6.0 mg), *Fr. F* (273.0 mg), *Fr. G* (554.0 mg), *Fr. H* (598.0 mg), and *Fr. I* (958.0 mg; MeOH-wash fraction). Previously, **1** and **2** were isolated from *Fr. D* and *Fr. H*, resp. [11]. *Frs. G*, *H*, and *I* were combined and subjected to a normal-phase CC (*Flash 25 + M*, *KP-Sil*, 25 × 150 mm, 40 g, *Biotage*) using a step gradient (step *1*, hexane/acetone from 100 : 0 to 80 : 20, 1200 ml; step *2*, hexane/acetone from 80 : 20 to 0 : 100, 600 ml) to afford *Frs. D1–D7* (598.9 mg), *Fr. D8* (297.3 mg), *Fr. D9* and *D10* (153.3 mg), *Fr. D11* (66.5 mg), *Fr. D12* (86.7 mg), *Fr. D13* (85.7 mg), *Fr. D14* (110.0 mg), *Fr. D15–D17* (433.2 mg), and *Fr. D18* (MeOH-wash fraction). *Fr. D8* was further separated by semi-prep. normal-phase HPLC (*Luna 5 µm Silica (2) 100 Å*, *Phenomenex*, 10 × 250 mm; hexane/^{i}PrOH, 99.2 : 0.8; flow rate, 4.7 ml/min; UV detection at 320 nm) to yield compounds **3** (t_R 15.4 min; 36.5 mg, 0.01995%) and **4** (t_R 17.0 min; 29.0 mg, 0.01585%). *Fr. D12* was further separated by semi-prep. normal-phase HPLC (*Luna 5 µm Silica (2) 100 Å*, *Phenomenex*, 10 × 250 mm; hexane/^{i}PrOH, 97.4 : 2.6; flow rate, 4.7 ml/min; UV detection at 320 nm) to furnish compounds **5** (t_R 19.5 min; 7.1 mg, 0.00388%), **6** (t_R 21.9 min; 25.3 mg, 0.01328%), and **7** (t_R 28.5 min; 6.8 mg, 0.00372%). *Fr. D14* was further separated by semi-prep. normal-phase HPLC (*Luna 5 µm Silica (2) 100 Å*, *Phenomenex*, 10 × 250 mm; hexane/^{i}PrOH, 95.0 : 5.0; flow rate, 4.7 ml/min; UV detection at 320 nm) to yield compounds **8** (t_R 22.1 min; 7.0 mg, 0.00383%) and **9** (t_R 29.0 min; 24.3 mg, 0.01328%).

Determination of Amounts of Compounds **1**–**9** *in CH_2Cl_2 Extracts.* Powdered roots of *E. transiliensis* (15 g) were extracted with 500 ml of CH_2Cl_2 for 1 d with a *Soxhlet* extractor (3 ×). The weights of extracts were 366.1, 386.1, and 379.7 mg, resp. The CH_2Cl_2 extract was subjected to HPLC (*ZORBAX SB-C18*, *Agilent*, 4.6 × 250 mm; MeOH/H_2O 75.0 : 25.0; flow rate, 1.0 ml/min; UV detection at 320 nm) to isolate **1** (t_R 29.8 min) and **2** (t_R 16.9 min). Also, the CH_2Cl_2 extract was subjected to normal-phase HPLC (*Luna 5 µm Silica (2) 100 Å*, *Phenomenex*, 4.6 × 250 mm; hexane/^{i}PrOH, 99.0 : 1.0; flow rate, 1.0 ml/min; UV detection at 320 nm) to isolate **3** (t_R 6.6 min) and **4** (t_R 7.3 min). Similarly, the CH_2Cl_2 extract was subjected to normal-phase HPLC (*Luna 5 µm Silica (2) 100 Å*, *Phenomenex*, 4.6 × 250 mm; hexane/^{i}PrOH, 94.0 : 6.0; flow rate, 1.0 ml/min; UV detection at 320 nm) to elute **5** (t_R 10.6 min), **6** (t_R 11.9 min), **7** (t_R 16.2 min), **8** (t_R 23.9 min), and **9** (t_R 27.5 min). The amounts of compounds **1**–**9** were calculated from their rel. peak area response values.

4-(2,2′-Bithiophen-5-yl)but-3-yne-1,2-diyl Diacetate (**3**). Yellow solid. ^{1}H-NMR (600 MHz, $CDCl_3$)[1]): 2.08 (*s*, Me(8″)); 2.12 (*s*, Me(6″)); 4.29 (*dd*, *J* = 7.2, 11.8, H_a–C(4″)); 4.40 (*dd*, *J* = 3.6, 11.8, H_b–C(4″)); 5.83 (*dd*, *J* = 3.6, 7.2, H–C(3″)); 7.00 (*m*, H–C(3)); 7.00 (*m*, H–C(4′)); 7.12 (*d*, *J* = 3.8, H–C(4)); 7.15 (*dd*, *J* = 1.0, 3.6, H–C(3′)); 7.22 (*dd*, *J* = 1.0, 5.1, H–C(5′)). ^{13}C-NMR (150 MHz, $CDCl_3$)[1]): 20.7 (C(8″)); 20.9 (C(6″)); 62.4 (C(3″)); 64.4 (C(4″)); 80.0 (C(1″)); 87.4 (C(2″)); 119.9 (C(5)); 123.3 (C(3)); 124.5 (C(3′)); 125.3 (C(5′)); 128.0 (C(4′)); 134.1 (C(4)); 136.4 (C(2′)); 139.8 (C(2)); 169.7 (C(5″)); 170.4 (C(7″)). EI-MS: 334.1 (M^+).

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[1]) Trivial atom numbering for assignments as indicated in *Fig. 1*.

4-[5-(Penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yne-1,2-diyl Diacetate (**4**). Yellow solid. ^{1}H-NMR (600 MHz, $CDCl_3$)[1]): 2.01 (*s*, Me(5′)); 2.07 (*s*, Me(8″)); 2.11 (*s*, Me(6″)); 4.27 (*dd*, *J* = 7.3, 11.9, H_a–C(4″)); 4.38 (*dd*, *J* = 3.6, 11.9, H_b–C(4″)); 5.80 (*dd*, *J* = 3.6, 7.3, H–C(3″)); 7.04 (*d*, *J* = 4.0, H–C(4)); 7.07 (*d*, *J* = 4.0, H–C(3)). ^{13}C-NMR (150 MHz, $CDCl_3$)[1]): 4.8 (C(5′)); 20.7 (C(8″)); 20.8 (C(6″)); 62.3 (C(3″)); 64.1 (C(3′)); 64.3 (C(4″)); 66.3 (C(1′)); 79.4 (C(1″)); 79.7 (C(2′)); 83.7 (C(4′)); 87.4 (C(2″)); 123.2 (C(5)); 124.6 (C(2)); 133.0 (C(4)); 133.5 (C(3)); 169.6 (C(5″)); 170.4 (C(7″)). EI-MS: 314.0 (M^+).

4-(2,2′-Bithiophen-5-yl)-2-hydroxybut-3-yn-1-yl Acetate (**5**). Yellow solid. ^{1}H-NMR (600 MHz, $CDCl_3$)[1]): 2.12 (*s*, Me(8″)); 4.28 (*m*, H_a–C(4″)); 4.28 (*m*, H_b–C(4″)); 4.82 (br. *s*, H–C(3″)); 6.99 (*m*, H–C(3)); 6.99 (*m*, H–C(4′)); 7.10 (*d*, *J* = 1.0, H–C(4)); 7.15 (*dd*, *J* = 3.5, 1.0, H–C(3′)); 7.22 (*dd*, *J* = 5.2, 1.0, H–C(5′)). ^{13}C-NMR (150 MHz, $CDCl_3$)[1]): 20.8 (C(8″)); 61.8 (C(3″)); 67.3 (C(4″)); 79.7 (C(1″)); 90.4 (C(2″)); 120.3 (C(5)); 123.4 (C(3)); 124.5 (C(3′)); 125.2 (C(5′)); 128.0 (C(4′)); 133.7 (C(4)); 136.4 (C(2′)); 139.5 (C(2)); 171.0 (C(7″)). EI-MS: 291.8 (M^+).

2-Hydroxy-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-1-yl Acetate (**6**). Yellow solid. ^{1}H-NMR (600 MHz, $CDCl_3$)[1]): 2.01 (*s*, Me(5′)); 2.11 (*s*, Me(8″)); 4.26 (*m*, H_a–C(4″)); 4.26 (*m*, H_b–C(4″)); 4.79 (br. *s*, H–C(3″)); 7.01 (*d*, *J* = 3.8, H–C(4)); 7.07 (*d*, *J* = 3.8, H–C(3)). ^{13}C-NMR (150 MHz, $CDCl_3$)[1]): 4.8 (C(5′)); 20.8 (C(8″)); 61.7 (C(3″)); 64.1 (C(3′)); 66.3 (C(1′)); 67.1 (C(4″)); 79.0 (C(1″)); 79.6 (C(2′)); 83.6 (C(4′)); 90.6 (C(2″)); 123.6 (C(5)); 124.3 (C(2)); 132.5 (C(4)); 133.5 (C(3)); 170.9 (C(7″)). EI-MS: 271.8 (M^+).

1-Hydroxy-4-[5-(penta-1,3-diyn-1-yl)thiophen-2-yl]but-3-yn-2-yl Acetate (**7**). Yellow solid. ^{1}H-NMR (600 MHz, $CDCl_3$)[1]): 2.01 (*s*, Me(5′)); 2.13 (*s*, Me(6″)); 3.86 (*m*, H_a–C(4″)); 3.86 (*m* , H_b–C(4″)); 5.63 (br. *s*, H–C(3″)); 7.04 (*d*, *J* = 3.8, H–C(4)), 7.07 (*d*, *J* = 3.8, H–C(3)). ^{13}C-NMR (150 MHz, $CDCl_3$)[1]): 4.8 (C(5′)); 20.9 (C(6″)); 64.1 (C(3′)); 64.3 (C(4″)); 65.5 (C(3″)); 66.3 (C(1′)); 79.4 (C(1″)); 79.7 (C(2′)); 83.6 (C(4′)); 88.1 (C(2″)); 123.3 (C(5)); 124.5 (C(2)); 132.9 (C(4)); 133.5 (C(3)); 170.0 (C(5″)). EI-MS: 271.8 (M^+).

4-(2,2′-Bithiophen-5-yl)but-3-yne-1,2-diol (**8**). Yellow solid. ^{1}H-NMR (600 MHz, $CDCl_3$)[1]): 3.76 (*dd*, *J* = 6.7, 10.4, H_a–C(4″)); 3.81 (*dd*, *J* = 3.6, 10.4, H_b–C(4″)); 4.68 (*m*, H–C(3″)); 6.99 (*m*, H–C(3)); 6.99 (*m*, H–C(4′)); 7.09 (*d*, *J* = 3.6, H–C(4)); 7.15 (*dd*, *J* = 0.7, 2.7, H–C(3′)); 7.22 (*dd*, *J* = 0.7, 5.1, H–C(5′)). ^{13}C-NMR (150 MHz, $CDCl_3$)[1]): 63.8 (C(3″)); 66.3 (C(4″)); 79.4 (C(1″)); 91.3 (C(2″)); 120.4 (C(5)); 123.3 (C(3)); 124.4 (C(3′)); 125.2 (C(5′)); 127.9 (C(4′)); 133.6 (C(4)); 136.4 (C(2′)); 139.4 (C(2)).

Compound **3** *Derived from Acetylation of* **8**. EI-MS: 333.8 (M^+). Methods for acetylation have been described in [19].

Larval Bioassays against Aedes aegypti. *A. aegypti* used in larvicidal bioassays originated from a laboratory colony maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, United States Department of Agriculture, Agriculture Research Service, Gainesville, Florida. This colony is maintained since 1952 using standard procedures [3]. Eggs were received and stored in our laboratory (Biological Field Station, The University of Mississippi, Abbeville, MS) until needed. Bioassays were conducted as described in [3] to determine the larvicidal activity of compounds **1**–**9** against 1-d-old *A. aegypti*. Eggs were hatched, and larvae were held in a room maintained at a temp. of 27 ± 2° with 60 ± 10% RH (relative humidity) under ambient fluorescent room light conditions. Five 1-d-old larvae were transferred to individual wells of a 24-well tissue culture plates in a 30–40-µl droplet of H_2O. 50 µl of larval diet of 2% slurry of 3 : 2 beef liver powder (*Now Foods*, Bloomingdale, Illinois), Brewer's yeast (*Lewis Laboratories Ltd.*, Westport, CT), and 1 ml of deionized H_2O were added to each well with a *Finnpipette* stepper (*Thermo Fisher*, FI-Vantaa). All the compounds to be tested were diluted with DMSO. After treatment application, the plates were swirled in clockwise and counterclockwise motions, front and back, and side to side five times to ensure even mixing of the chemicals. Larval mortality was recorded 24- and 48-h post treatment. Larvae were deemed dead if they showed no movement in the well after being prodded with a pipette tip. Permethrin (46.1% *cis*/53.2% *trans*, *Chemical Service*, West Chester, PA) was used as positive control. A series of five dosages were used in each treatment to get a range of mortality between 0 and 100%. Treatments were replicated ten times for each compound.

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